A STUDY OF THE ESCAPE OF CERCARIAE FROM THEIR SNAIL HOSTS*

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During the summer of 1920 while studying at the University of Michigan Biological Station at Douglas Lake, Michigan, I undertook a series of experiments on the escape of cercariae from their snail hosts. The object of these experiments was to determine the numbers of cercariae escaping from snails infested with trematodes and the times at which these cercariae madè their escape. Four species of cercariae were studied, viz.: (1) *Cercaria elephantis* Cort (1917), a schistosome cercaria with eyespots from *Planorbis trivolvis* Say; (2) *Cercaria emarginatae* Cort (1917), a forked tailed cercaria with a pharynx from *Lymnaea emarginata angulata* Sowerby; (3) an undetermined echinostome cercaria from *Physa ancillaria parkeri* Currier, and (4) an undetermined stylet cercaria from the same host. Most of the studies were made on the escape of *Cercaria elephantis* from *Planorbis trivolvis,* the small number of experiments on the other three species of cercariae being carried out near the end of the work to obtain some comparative data.

In order to determine which snails were infested with the cercariae the following simple method was used: A collection of about 100 specimens of a given species of snail would be brought into the laboratory late in the afternoon. These snails were then divided into groups of four or five and the groups placed in separate wide mouthed six or eight ounce bottles about one-third full of water. The next morning some of the water from these bottles was examined in a watch glass under a dissecting microscope. If any cercariae had escaped they could be easily seen and the groups containing infested snails determined. These groups were then redivided, only one snail being placed in a bottle, and by further examinations the individual snails infested were determined. Examination of the water from negative groups was repeated late in the afternoon to catch any cercariae which escaped only in the daytime. When a high percentage of snails were infested no preliminary division was made, the snails being immediately placed in separate bottles.

With the infested snails isolated it was possible to pour off the water from around them at definite intervals and count the cercariae given off during certain definite periods. A new supply of water was

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TABLE 1.—DATA ON THE ESCAPE OF CERCARIA ELEPHANTIS FROM PLANORBIS TRIVOLVIS

Day	Hour	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
July													
4	11 a m - 3:30 p m	146	0	0									
4	3:30 p m - 9 p m	5	420	121									
4-5	9 p m - 8 a m	3	*	*									
5	8 a m - 1 p m	1000±	8	4									
5	1 p m - 7 p m	186	104	25									
5-6	7 p m - 9 a m	5	150	164									
6	9 a m - 12 n	43	3	3									
6	12 n - 3 p m	219	0	0									
6	3 p m - 6 p m	19	0	0	43	4	47						
6	6 p m - 9 p m	5	6	1	7	0	3						
6-7	9 p m - 7 a m	5	129	5	15	60	6						
7	7 a m - 9 a m	20	26	3	134	11	21						
7	9 a m - 12 n	653	2	1	127	1	150						
7	12 n - 3 p m	167	3	0	70	0	85						
7	3 p m - 5 p m	32	33	2	23	6	61						
7	5 p m - 7 p m	4	254	3	1	52	71						
7	7 p m - 9 p m	15	610	161	2	166	4						
7-8	9 p m - 7:30 a m	1	295	125	75	125	43						
8	7:30 a m - 9 a m	720	25	27	220	18	113						
8	9 a m - 12 n	882	7	6	180	5	95						
8	12 n - 3 p m	450	2	0	104	0	93						
8	3 p m - 6 p m	41	60	0	77	0	61						
8	6 p m - 9 p m	34	296	135	12	64	16						
8-9	9 p m - 12:30 a m	1	392	46	12	70	7						
9	12:30 a m - 3:15 a m	0	40	12	0	24	1						
9	3:15 a m - 6:45 a m	0	14	2	1	5	0						
9	6:45 a m - 9 a m	55	2	3	211	1	82						
9	9 a m - 12 n	490	0	2	213	0	108						
9	12 n - 3 p m	797	0	0	98	1	110						
9	3 p m - 6 p m	50	30	1	37	0	82						
9	6 p m - 9 p m	11	489	247	7	31	6						
9-10	9 p m - 12:15 a m	0	146	46	1	40	2						
10	12:15 a m - 3:30 a m	0	4	75	0	21	0						
10	3:30 a m - 6:45 a m	0	9	5	0	0	0						
10	6:45 a m - 9 a m	75	4	2	140	0	14						
10	9 a m - 12 n	1218	0	3	111	0	114						
10	12 n - 3 p m	559	2	1	346	0	47						
10	3 p m - 6 p m	35	186	60	76	5	85						
15	5:30 p m - 9 p m	....	82	6	5	....	....	75	35	42	0	1	1
15	9 p m - 12 m	....	36	23	2	....	....	12	10	32	0	0	0
16	12 m - 3 a m	....	11	7	0	....	....	0	0	0	0	0	0
16	3 a m - 6 a m	....	3	1	2	....	....	0	0	0	1	0	0
16	6 a m - 9 a m	....	1	1	7	....	....	0	0	0	0	1	0
16	9 a m - 12 n	....	5	1	120	....	....	1	0	0	0	43	7
16	12 n - 3 p m	....	10	1	61	....	....	2	0	0	0	91	25
16	3 p m - 6 p m	....	151	44	31	....	....	61	18	38			
16	6 p m - 9 p m	....	110	226	13	....	....	307	269	565	....	3	4
16	9 p m --12 m	....	24	154	0	....	....	10	34	2	....	0	0
17	12 m - 3 a m	....	4	40	0	....	....	2	11	0	....	0	0
17	3 a m - 6 a m	....	5	6	1	....	....	....	5	....	....	0	0
17	6 a m - 9 a m	....	....	....	143	....	....	....	....	....	....	27	20
17	9 a m - 12 n	....	....	....	98	....	....	....	....	....	....	56	37
17	12 n - 3 p m	....	....	....	25	....	....	....	....	....	....	49	82
17	3 p m - 6 p m	....	....	....	57	....	....	....	....	....	....	15	5
19	7:30 a m - 9 a m	....	....	....	112	....	....	....	....	....	....	62	
19	9 a m - 12 n	....	....	....	119	....	....	....	....	....	....	90	
19	12 n - 1 p m	....	....	....	1	....	....	....	....	....	....	70	
19	1 p m - 6 p m	....	....	1	....	....	....	....	....	....	1		
19	6 p m - 9 p m	....	....	1	....	....	....	....	....	....	114		
19	9 p m - 12 m	....	....	6	....	....	....	....	....	....	145		
20	12 m - 7:30 a m	....	....	11	....	....	....	....	....	....	5		
Aug.													
5	9 a m - 12 n	....	....	0	15	....	....	....	....	3	....	12	
5	12 n - 3 p m	....	....	0	29	....	....	....	....	1	....	6	
5	3 p m - 6 p m	....	....	0	30	....	....	....	....	2	....	5	
5	6 p m - 9 p m	....	....	0	1	....	....	....	....	4	....	0	
5	9 p m - 12 m	....	....	2	0	....	....	....	....	7	....	0	
6	12 m - 3 a m	....	....	5	0	....	....	....	....	0	....	1	
6	3 a m - 6 a m	....	....	6	0	....	....	....	....	0	....	0	
6	6 a m - 9 a m	....	....	0	9	....	....	....	....	1	....	2	
6	9 a m - 12 n	....	....	0	17	....	....	....	....	3	....	7	
6	12 n - 3 p m	....	....	0	21	....	....	....	....	1	....	8	
6	3 p m - 6 p m	....	....	0	6	....	....	....	....	1	....	1	
6	6 p m - 9 p m	....	....	15	9	....	....	....	....	2	1		
6-7	9 p m - 7:30 a m	....	....	14	7	....	....	....	....	9	0		

* Not counted.

then poured into the bottles and the process repeated as desired. Of course it is possible that all cercariae free in the water would not be poured out, but the bottles were thoroughly rinsed each time the water was poured off and in all cases the treatment was the same, so that this hardly seems to be an important source of error. For counting enough formalin was mixed with the water to kill the cercariae and they were counted in a ruled Syracuse watch glass under a dissecting microscope. By this method it can be determined how many cercariae escape from a particular snail during a given period and whether they escape at regular intervals or in cycles.

The Numbers of Cercariae Escaping from Snails

The data obtained in regard to the escape of *Cercaria elephantis* from *Planorbis trivolvis* are included in Table 1. On examining this table, one is struck with the large numbers of cercariae which must escape from an infested snail of this species during the course of a summer. The data do not give accurate information on this point, since the length of the period of cercaria-production in any one snail is not known. The experiments give records of cercariae escaping from snails III and IV for periods extending over about a month. The record is more complete for snail IV, from which cercariae escaped as follows: (See Table 1)—July 7, 372; July 8, 680; July 9, 568; July 10, 673; July 16, 234; July 17, 324; July 21, 447; July 22, 263; August 6, 69. The small number of cercariae escaping from this snail on August 6 may indicate that the period of cercaria-production was nearing its end. This conclusion does not necessarily follow, however, since snail IV by August 6, was in an advanced stage of starvation, which may have influenced the number of cercariae produced. These nine records which are scattered through the month give an average per day of 400 cercariae escaping. For the period of 31 days, during which this snail was observed, this would give a total of about 12,400 cercariae, which may be taken as an approximation of the number of cercariae which escaped from this snail during the month. A single month certainly does not represent the total period of cercaria production in this snail, since the number was at its height when the observations started.

Further, in some of the other snails the numbers of cercariae escaping each twenty-four hours were much larger, as for example snails I and II (see tables 1 and 3). These figures give some idea of the enormous numbers of cercariae of this species which would escape into a body of water containing only a relatively small number of infested individuals of *Planorbis trivolvis*.

When the records of the escape from their snail hosts of the other three species of cercariae studied are examined, the numbers involved

are even more striking (see Table 2). For example, in the case of snail IV of the stylet cercaria series over 3,000 cercariae escaped on August 14, and in the *Cercaria emarginatae* series over 5,000 cercariae escaped from snail V on August 16 (see Table 2). These findings emphasize the enormous reproductive wastage in the development of cercariae in the digenetic trematodes, which is necessary on account of the great difficulty that they experience in reaching their final hosts.

TABLE 2.—DATA ON ESCAPE FROM SNAIL HOSTS OF THREE CERCARIAE

Day Aug.	Hour	Echinostome Cercaria				Stylet Cercaria				Cercaria emarginatae					
		I	II	III	IV	I	II	III	IV	I	II	III	IV	V	VI
12	10 a m - 2 p m	446	535	251	233	272	350	673	628	18	0	148	425		
12	2 p m - 6 p m	157	200	177	275	242	270	324	430	234	3	67	475		
12	6 p m - 10 p m	30	12	5	17	414	269	244	1061	213	6	142	28		
12-13	10 p m - 6 a m	17	1	3	0	255	309	765	377	95	166	664	14		
13	6 a m - 10 a m	189	12	4	5	236	407	822	449	192	18	211	266		
14	5:15 a m - 8:15 a m	...	49	...	...	...	...	...	1020	...	...	...	65		
14	8:15 a m - 11:15 a m	...	55	...	...	...	...	...	840	...	...	...	165		
14	11:15 a m - 2:15 p m	...	249	...	...	...	...	...	1323	...	...	...	109		
14	2:15 p m - 5:15 p m	...	116	...	...	...	...	...	687	...	...	...	277		
14	5:15 p m - 9:45 p m	...	153	...	...	...	...	...	490	...	...	...	146		
14-15	9:45 p m - 9 a m	...	1	...	...	...	...	...	564	...	...	...	0		
15	4 p m - 8 p m	...	...	166	477	...	...	...	....	102	4	151	82	19	252
15	8 p m - 12 m	...	...	0	15	...	...	...	....	240	67	755	8	6	15
16	12 m - 4 a m	...	...	1	8	...	...	...	....	149	37	86	0	0	8
16	4 a m - 7 a m	...	...	1	2	...	...	...	....	119	2	64	49	1	10
16	7 a m - 9 a m	...	...	237	63	...	...	...	....	169	5	279	84	3720±	927
16	9 a m - 11 a m	...	...	131	133	...	...	...	....	13	0	58	10	1440±	238
16	11 a m - 1 p m	...	...	314	182	...	...	...	....	3	2	64	7	184	32
16	1 p m - 3 p m	...	...	75	457	...	...	...	....	3	0	5	0	71	8
16	3 p m - 5 p m	...	...	81	192	...	...	...	....	0	0	0	0	13	0
16	5 p m - 7 p m	...	...	5	68	...	...	...	....	0	0	2	208	6	2

TABLE 3.—SUMMARY OF THE NUMBERS OF CERCARIA ELEPHANTIS ESCAPING FROM SIX SPECIMENS OF PLANORBIS TRIVOLVIS

Periods	I	II	III	IV	V	VI
July 6, 6 p m to July 7, 7 p m.....	886	453	15	377	130	397
July 7, 7 p m to July 8, 6 p m.....	2109	945	319	558	314	409
July 8, 6 p m to July 9, 6 p m.....	1427	774	201	584	165	406
July 9, 6 p m to July 10, 6 p m.....	1896	840	439	671	97	268
Total.....	6318	3012	974	2190	706	1480

Variations in Different Snails of the Same Species

In the twelve specimens of *Planorbis trivolvis* from which the escape of *Cercaria elephantis* was recorded, there is seen to be a considerable variation in the numbers of cercariae coming out from the different individuals (see Table 1). This point is clearly illustrated from the records of snails I to VI for the four day period from 6 P. M., July 6, to 6 P. M., July 10. The summary of these data (Table 3) shows a great variation in the output of cercariae from these six snails, which during this period were subjected to exactly the same conditions.

That there is a definite relation between the numbers of sporocysts present in a given snail and its output of cercariae was shown by dissections of snails I, V and VI of this series. The comparison of the conditions of the livers of snails I and V, which represent the extremes of cercariae output, was most interesting. Both of these snails were cut open in such a way that the liver was not broken. In both cases the sporocysts were limited to the liver. The liver of snail I was of a uniform pale yellow color, while that of V had a mottled appearance, dark patches being interspersed among the yellow. When the livers of these two snails were carefully examined, it was found that in I all the tissue was packed with a dense mass of tangled sporocysts which had practically replaced all the liver substances. The number of the sporocysts was so great and they were so densely packed and tangled that it was impossible to count them. In snail V it was found that the lighter areas were made up of a large number of sporocysts, but that they were not so densely packed or tangled and that the dark areas between were composed of liver substance which had not been invaded by sporocysts. An estimate of the numbers of sporocysts in these two snails gave I about ten times as many as V, which agrees with the difference in the number of cercariae which escaped. The dissection of snail VI showed that its liver had a somewhat mottled appearance, as in V, but that the dark areas were much smaller, and that the crowding of sporocysts was not nearly as great as I. Therefore VI showed an intermediate condition between I and V.

In a later dissection an attempt was made to count the number of sporocysts in the liver of snail VIII. This count gave approximately 200 sporocysts in this one snail. In the period just before the dissection was made from 3 P. M., July 16, to 6 A. M., July 17, which apparently represented a complete 24-hour cycle for this snail, 337 cercariae were given off. The data given above show that it is possible to correlate the numbers of cercariae given off from any snail with the numbers of sporocysts in its liver.

While the data available are not so extensive the individual variations in the output of the other cercariae studied are just as striking as in the case of *Cercaria elephantis* (see Table 2). For example, in the stylet cercaria series there is a big difference between I and IV. In the case of *C. emarginatae* there is the most striking variation in the whole series in the comparison of II, with a daily output of between 100 and 200, and V, of which is given a record of over 5,000 cercariae given off in one 24-hour period (see Table 2).

Variations in Cercariae Escaping from the Same Snail

From Table I it will be seen that there was a considerable variation on different days in the number of cercariae escaping from the same snail. Of course, variations would be expected in the numbers of

cercariae coming to maturity in the liver of a snail from day to day, and there possibly might have been a small variation due to errors in counting. It is further evident that in the cercaria-producing period of a given infested snail there would be an early period of small cercaria production and a period near the end when the numbers would be diminished. It also seems probable that the great reduction in the cercariae escaping from snails III, IV, IX and XI after August 5 was due to the fact that these snails were badly starved, it being difficult to give them sufficient food under the conditions of the experiment.

It was found also that the temperature of the environment very significantly affected the numbers of cercariae escaping from the snails. On the night of July 5 the temperature dropped to 39.5 F. and the morning of July 6 was cold and rainy. On this day, from 9 A. M., to 6 P. M., only 281 cercariae are recorded as escaping from snail I, as compared with between 1,000 and 2,000 on warmer days. During the other days of the experiment there was a fairly uniform temperature, and no other such marked variations were noted.

To test by experiment the effect of low temperatures on the escape of the cercariae the bottles containing snails IV, XI and XII were kept in water from a well at a temperature ranging from 58 F. to 63 F. from 8:30 P. M., July 19, to 6 P. M., July 20. As shown on the records for July 17 (Table 1), the cercariae were accustomed to escape from these three snails from 6 A. M. to 6 P. M. Therefore, these snails had been kept at low temperatures for the whole night before their cycle usually commenced and during one complete cycle. Examinations were made during this day and it was found that the escape of the cercariae was almost completely inhibited (see records for July 20 on Table 4). From 8 A. M. to 6:45 P. M. on June 20 only 22 cercariae escaped from snail IV; 18 from XI, and 5 from XII, as compared with the normal cycle on July 17, when during approximately the same period 323 cercariae were recorded from IV, 147 from XI and 94 from XII. At 6 P. M. on July 20, the bottles were removed from the cold water and as the temperature at this time was 74 F. they soon warmed up. This increase in temperature was immediately reflected in the escape of cercariae, as is shown by the records from 6:45 P. M. to 9:30 P. M. and from 9:30 P. M. to 6:30 A. M. (see Table 4 records for July 20 and 21), since in all three of these snails there was a considerable number of cercariae found at a time when in their normal cycle practically no cercariae would escape. The cycle of escape of cercariae then resumed its daylight character, as is shown by the records from July 21 and 22. This experiment showed that a temperature only slightly below normal will inhibit the escape of cercariae from their snail hosts. It therefore seems probable that temperature is an important factor in regulating the escape of cercariae from their snail hosts. The investigations just

recorded are merely suggestive and should be carried out much further for various species of cercariae. Judged from these findings, however, it may well be that the infectivity to man of waters in which live snails that harbor the cercariae of the human schistosomes, may be found to be profoundly influenced by temperature.

The Time at Which Cercariae Escape from the Snails

Early in my work on the escape of *Cercaria elephantis* from *Planorbis trivolvis,* I found that the escape of the cercariae did not extend over the whole 24 hours, but that there were periods when they escaped in numbers followed by periods during which none escaped. In other words, the escape came in waves recurring every 24 hours and covering only a part of this period. Perhaps the most surprising finding was that the time of these waves differed in different snails, in some occurring in the daytime and in others at night. Further, it was

TABLE 4.—NUMBER OF CERCARIAE ESCAPING FROM THREE SNAILS, KEPT AT A TEMPERATURE RANGING BETWEEN 58° AND 63° F., FROM 8:30 P. M., JULY 19, TO 6 P. M., JULY 20

Day	Hour	IV	XI	XII
July 20	8 a m - 12 n	14	11	2
July 20	12 n - 3 p m	7	2	2
July 20	3 pm - 6:45 p m	1	5	1*
July 20	6:45 p m - 9:30 p m	88	69	59
July 20–21	9:30 p m - 6:30 a m	82	71	15
July 21	6:30 a m - 9 a m	86	41	31
July 21	9 a m - 12 n	121	100	57
July 21	12 n - 3 p m	106	96	61
July 21	3 p m - 6:45 p m	118	115	163
July 21	6:45 p m - 9:30 p m	16	27	16
July 21–22	9:30 p m - 6:45 a m	17	21	7
July 22	6:45 a m - 9:30 a m	74	62	28
July 22	9:30 a m - 12 n	30	110	50
July 22	12 n - 3 p m	49	67	87

* End of period in cold water.

found that the time of these waves suffered but slight change on the different days on which a given snail was studied. For example, in snail I the escape of the cercariae came in the daytime and was pretty largely limited to a period from 6 A. M. to 6 P. M., with the largest numbers escaping from 9 A. M. to 3 P. M. This cycle remained constant during the seven days in which this form was studied. In II the period extended from about 3 P. M. to midnight; in III from about 6 P. M. to 3 A. M.; in IV from about 6 A. M. to 6 P. M.; in V from about 6 P. M. to 3 A. M.; and in VI from about 6 A. M. to 6 P. M. In the other snails of this species studied the same type of cycle of escape of cercariae with its variations in different specimens can be traced (see Table 1). While there are certain slight variations in these waves, their constancy even over a considerable period is very striking. I have no explanation to offer for this phenomenon, which can only have its origin in a periodicity of development of the cercariae themselves.

This same type of periodicity is manifested by *Cercaria emarginatae* in its escape from *Lymnaea emarginata angulata,* as can be seen from Table 2. *C. emarginatae* is also a forked-tailed cercaria, although the presence of a pharynx places it in a different group from *C. elephantis.*

The studies on the echinostome cercaria and the stylet cercaria were made chiefly to compare their cycles with the two forked-tailed species. The cycles of escape of these cercariae from their snail hosts were found to be quite different (see Table 2). In the echinostome species the escape of the cercariae was almost entirely limited to the daytime. In this connection it is interesting to note that echinostome cercariae show a very striking positive reaction to light. The stylet form studied escaped from its snail host during the whole 24 hours, although there was a distinctly smaller number which escaped during the night than during the day (see Table 2).

The results of the experiments outlined above on the escape of cercariae from their snail hosts are in many cases more suggestive than conclusive. They certainly show, however, that further work along this line will be profitable. Since the purpose of the free life of the cercariae is entrance into the definitive host, it is very possible that a study of this same problem for the cercariae of the human trematodes may shed light on the general problem of human infestation and give data of value in control.

Literature Cited

Cort, W. W. 1917.—Homologies of the Excretory System of the Forked-Tailed Cercariae. Jour. Parasit., 4: 49-57.